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[COMMUNICATION]

Identification of Intracellular Localization of Laminin in the Rat Anterior Pituitary

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ABSTRACT—The intracellular localization of laminin of endocrine cells in the rat anterior pituitary was examined by biochemical methods and electron-microscopical immunocytochemistry. The secretory granules of the anterior pituitary cells were separated. And the granules were immunostained by the post-embedding method using antiserum against laminin. Laminin was detected on the surface of the membrane of separated granules. On the other hand, the granules were immunoblotted using antiserum and monoclonal antibody against laminin. The antiserum reactive with both A-chain and B-chains of the antigen (mouse EHS sarcoma laminin) cross-reacted with only the B-chain(s) of the secretory granules, but the monoclonal antibody showed no cross-reactivity. These results suggest that laminin in the anterior pituitary cells may exist as B-chain singly in the secretory granules and it may be different from mouse EHS sarcoma laminin in structure.

INTRODUCTION

Laminin [1, 2], one of the major components of the basement lamina, has been immunocytochemically observed in various organs, and it has also been proved to play a role in cell attachment, migration, proliferation and differentiation. In the pituitary gland, Tougard *et al.* have detected immunocytochemically that laminin exists on the basement lamina and the parenchymal cells, especially gonadotrophs of the anterior pituitary gland [3]. Thereafter, similar evidence has been reported by others [4–9], but obtained results including the identification of the positive cells and the intracellular site of laminin are still debatable. Besides, in these reports examinations have been conducted only by immunocytochemical methods for the identification of laminin, and there is no information about its intracellular localization using other methods. It is important to clarify the intracellular localization of laminin in the immunoreactive pituitary cells (laminin positive cells). In the present study, we separated the secretory granules from the anterior pituitary of rats and identified the localization of laminin in the secretory granules by both the method of immunoblotting and that of electron-microscopical immunocytochemistry.

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MATERIALS AND METHODS

Animals

Wistar-Imamichi male rats (60 day-old) were obtained from the Imamichi Institute for Animal Reproduction. One hundred rats for granule separation and immunoblotting, and six for light-microscopical immunocytochemistry were used.

Antibodies

The following antibodies were used for detecting laminin; antiserum against mouse EHS sarcoma laminin (Advance, Tokyo, ×1000–32000 in dilution) and monoclonal antibody against human placental laminin (Iwaki Glass, Tokyo, ×1000). Antiserum against rat LH-β (NIDDK, ×8000) was used for immunocytochemical identification of LH cells. Antiserum against bovine type IV collagen

(Advance, ×4000) was used for checking the contamination of basement lamina in the granule solution. The ABC kit (Vector Lab.) was used for the further process of immunocytochemistry and immunoblotting, after using the first antibodies. And the gold-colloid (15 nm diameter) conjugated antibody (Ig-G) against rabbit Ig-G (E. Y. Lab.) was used as the second antibody for electron-microscopical immunocytochemistry. The specificity of the antisera for laminin and type IV collagen was reported by Aihara *et al.* [10]. The antisera for mouse laminin and bovine type IV collagen sufficiently cross-react with rat laminin or type IV collagen respectively. And the adsorption-test of the antisera for mouse laminin and rat LH-β had done by Kusaka *et al.* [9]. Enzyme-linked immunosorbent assay (ELISA) [11, 12] showed that the monoclonal antibody strongly reacted with human placental laminin, and it reacted also with both subunits of mouse EHS sarcoma laminin but only slightly.

Immunocytochemistry

After decapitation, the pituitary glands were removed and fixed in formolsublimate solution and embedded in Paraplast. Three μm thick serial sections were then prepared, deparaffinized, and treated with pepsin [13]. Two serial sections were stained by immunoperoxidase method (ABC method) [14] with the antisera either against laminin (×32,000) or LH-β respectively. The stained sections were counterstained with Hematoxylin solution.

Separation

A modified method reported by Costoff *et al.* [15] was used for separation of the secretory granules from the rat anterior pituitary. After decapitation, the anterior glands were removed and homogenized. The nuclei were excluded by a centrifuge and the supernatant was filtrated, loaded on 5–50% Ficoll 400 (Pharmacia), 0.25 M saccharose, 0.5 mM EDTA (pH 7.2), and centrifuged (100,000×g, 2 hr.). After the centrifugation, all the zones containing the hormone granules were collected and centrifuged (100,000×g, 1 hr.). The precipitate was then suspended with PBS and sonicated. The solution thus obtained was used as the secretory granule solution. No practical contamination of basement lamine was observed in the granule solution since the solution immobilized on the nitrocellulose membrane did not react immunocytochemically with the antiserum against type IV collagen, a major component of basement lamina.

Electron microscopy

The purity and immunoreactivity of the separated secretory granules were checked by the post-embedding method [16, 17]. The granules were fixed in picric acid-paraformaldehyde solution [18] and osmium tetroxide solution [19], and embedded in Quetol 651 (Nisshin EM, Tokyo) by a modified method reported by Kushida [20]. The thin sections were reacted with the antiserum against laminin (×1000) or normal rabbit serum (NRS), followed by the gold-colloid conjugated 2nd antibody. They were then refixed in the same fixative. No electron staining was employed. Again, no practical contamination of other cellular components was visually observed (Fig. 2).

Immunoblotting

The granule solution was separated by SDS-PAGE [21] (gel concentration: 6%) and silver-stained (Silver Stain KANTO, Kanto Chemical, Tokyo) or immunoblotted [22], by the use of ABC method. Antiserum against mouse laminin (× 4000) and monoclonal antibody against human laminin were used as the first antibody. And the silver-stained gel was analyzed by a chromatoscanner (Shimadzu CS-9000).

RESULTS

The result of light-microscopical immunocytochemistry is shown in Figure 1. The antiserum against EHS laminin reacted with the capillary basement lamina and some of parenchymal cells. The laminin positive cells (Fig. 1a) were corresponded with LH cells (Fig. 1b).

Electron-microscopical observation revealed that the separated granule fraction contained practically only the secretory granules (Fig. 2), and the antiserum against EHS laminin more or less reacted with the granules despite of their densities

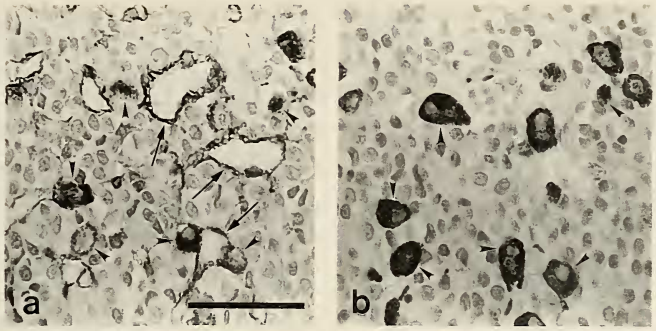


FIG. 1. The adjacent sections of the anterior pituitary were immunostained by antiserum against mouse lamin in (a) or rat LH-β (b). arrowhead: laminin positive cell, arrow: capillary, Bar: 50 μm.

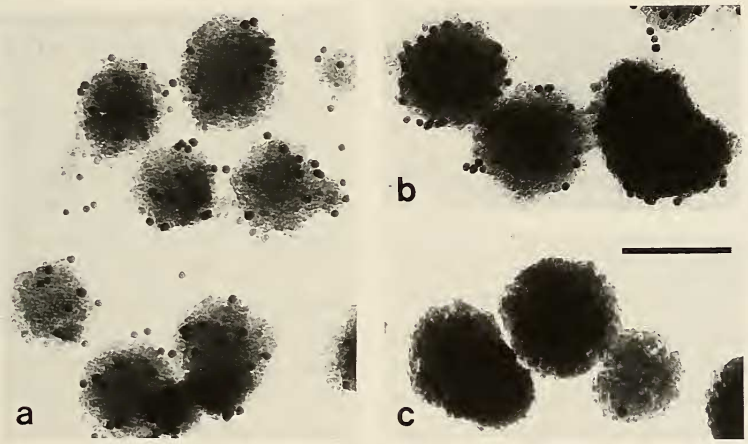
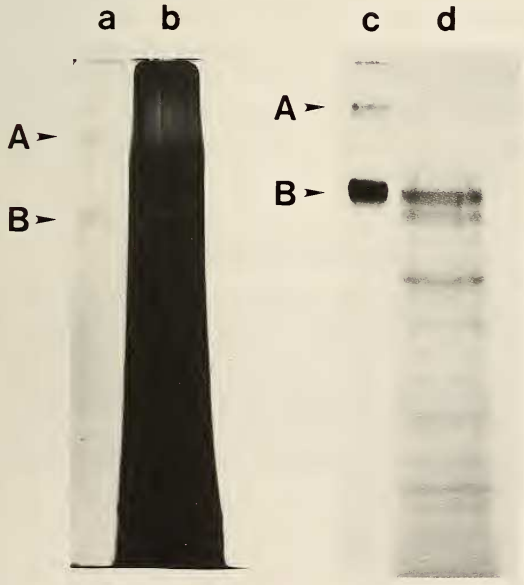


FIG. 2. Electron-microscopical immunocytochemistry of the secretory granule solution. Antiserum against mouse laminin reacted with the granules. a: antiserum against laminin (low density), b: antiserum against laminin (high density), c: NRS, Bar: 200 nm.



(Fig. 2a, b).

The results of silver-staining and immunoblotting using EHS laminin antiserum are shown in Figure 3. The B-chains were not separated in this electrophoresis condition; therefore two bands of mouse laminin (the A-chain and B-chains bands) were stained (Fig. 3a, c). The lane of electrophoresed granule solution was silver-stained almost entirely (Fig. 3b), in which immunoblotting detected only B-chains but not A-chain (Fig. 3d). In addition, some bands of lower molecular weight were stained.

FIG. 3. Mouse laminin (a, c: 0.18 μg) and the secretory granules (b, d: 85.6 μg) were silver-stained (a, b) and immunostained by antiserum against mouse EHS sarcoma laminin (c, d). A: A-chain, B: B-chains.

Fig. 4. Mouse laminin (a: 1.5 μg) and the secretory granules (b: 85.6 μg) were immunostained by monoclonal antibody against human placental laminin. A: A-chain, B: B-chains.

The monoclonal antibody was able to detect both A-chains and B-chains of mouse EHS laminin (Fig. 4a), but neither A- nor B-chains of the secretory granules were detected (Fig. 4b). Again, some bands with lower molecular weight were stained.

DISCUSSION

The granule preparation seemed to include almost all kinds judging from their specific gravities and sizes [15]. Almost all the granules were laminin positive as far as examined, although stainabilities varied from granule to granule. This result agreed with the demonstration of Vila-Porcile *et al.* [5] in which all the secretory cells embedded in an electron-microscopical thin section, were entirely stained. Light-microscopical observation, however, showed that only LH cells were laminin positive that accorded with the result obtained by Tougard *et al.* [3]. The reason of the discrepancy may be explained as follows; LH granules contains more laminin than others, thus the light-microscopical method is not sensitive enough for the detection of intracellular laminin other than LH cells, nevertheless electron-microscopical method can detect such the slight amount of the antigen because of intracellular localization.

On the molecular study by immunoblotting, a polyclonal antiserum against mouse EHS sarcoma laminin reacted with the electrophoretically B-chain equivalent portion of laminin and several smaller molecules of the secretory granules but not with the A-chain, although it reacted with both the A-chain and the B-chains of the antigen. On the other hand, no laminin equivalent bands were stained with a monoclonal antibody against the human laminin that could stain both chains of EHS laminin. Many smaller molecules were again stained with the antibody.

A-chain equivalent molecules were not detected at any time in the present experiment. These results suggest that there exist laminin-like molecules different in structure from the laminin of the basement lamina in the secretory granules of the rat anterior pituitary. We now tentatively assume that these consist of a single or double subunit of B-chain-like molecules. Many immunopositive molecules smaller than B-chain molecules were detected by the two antibodies used presently. It is however still unknown if they are fragments of the molecule or they are physiologically synthesized in the pituitary cells.

Recently, Hunter *et al.* reported that a type of laminin (s-laminin) different from that of the basement lamina exists in normal rat tissues (e.g. synaptic cleft, glomerular) [23]. The secretory granule laminin (g-laminin) presently reported may also have been different from that of the basement lamina.

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REFERENCES

1 Timpl, R., Rohde, H., Robey, P. G., Rennard, S. I., Foidart, J. M. and Martin, G. R. (1979) J. Biol. Chem., **254**: 9933–9937.

2 Beck, K., Hunter, I. and Engel, J. (1990) FASEB J., **4**: 148–160.

3 Tougard, C., Louvard, D., Picart, R. and Tixier-Vidal, A. (1985) In vitro Cell Develop. Biol., **21**: 57–61.

4 Holck, S., Albrechtsen, R. and Wewer, U. M. (1987) Lab. Invest., **56**: 481–488.

5 Vila-Porcile, E., Picart, R., Tixier-Vidal, A. and Tougard, C. (1987) J. Histochem. Cytochem., **35**: 287–299.

6 Vila-Porcile, E., Picart, R., Olivier, L., Tixier-Vidal, A. and Tougard, C. (1988) Cell Tissue Res., **254**: 617–627.

7 Leardkamolkarn, V., Heck, L. W. and Abrahamson, D. R. (1989) Cell Tissue Res., **257**: 587–596.

8 Nunez, E. A., Pomeranz, H. D., Gershon, M. D. and Payette, R. F. (1990) Anat. Rec., **226**: 471–480.

9 Kusaka, S., Yamashita, S., Yamaguchi, S. and Kusunoki, S. (1987) Zool. Sci., **4**: 991 (Proceeding).

10 Aihara, M., Izawa, H., Kumagai, N., Ishida, H., Ogino, Y., Kimura, Y., Yashiro, T. and Suzuki, T. (1988) J. Jpn. Plastic Reconstruct. Surgery, **8**: 410–418.

11 Engvall, E. and Perlmann, P. (1971) Immunochem., **8**: 871–874.

12 Miles, L. E. M. and Hales, C. N. (1968) Nature, **219**: 186–189.

13 Burns, J., Dixon, A. J. and Woods, J. C. (1980) Histochem., **67**: 73–80.

14 Hsu, S-M., Raine, L. and Fanger, H. (1981) J. Histochem. Cytochem., **29**: 577–580.

15 Costoff, A. and McShan, W. H. (1969) J. Cell Biol., **43**: 564–574.

16 Faulk, W. P. and Taylor, G. M. (1971) Immunochem., **8**: 1081–1083.

17 Roth, J., Ravazzola, M., Bendayan, M. and Orci, L. (1981) Endocrinol., **108**: 247–253.

18 Zamboni, L. and De Marrino, C. (1967) J. Cell Biol., **35**: 148A (Abstract)

19 Millonig, G. (1962) In "Electron Microscopy. Vol. 2 (Fifth international congress for electron microscopy)" Ed. by SS Breese Jr., Academic Press, New York, pp. 8.

20 Kushida, H. (1974) J. Electron Microscopy, **23**: 197.

21 Laemmli, U. K. (1970) Nature, **227**: 680–685.

22 Burnette, W. N. (1981) Anal. Biochem., **112**: 195–203.

23 Hunter, D. D., Shah, V., Merlie, J. P. and Sanes, J. R. (1989) Nature, **338**: 229–234.